Flavour Compounds in Cheese (Review)

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Abstract

Cheese flavour development is a complex process in which enzymes from milk, rennet, starter cultures and secondary flora are involved in the degradation of milk proteins, fat and carbohydrates. Variations in on non-starter lactic aid bacteria (NSLAB) and derived compounds depend on cheese variety, processing and ripening condition. Starter has an important role during its ripeness due to (1) the production of lactic acid, which together with the rennet causes the curd to form, acts as a preservative and contributes to the acid flavour of the cheese, (2) metabolism of citric acid, which is widely regarded as essential for flavour production, (3) breakdown of the protein (4) some contribution to the breakdown of the diglycerides formed from the milk triglycerides by the lipoprotein lipase from the milk (5) the breakdown of hippuric acid to benzoic acid. Also, enzymes playing an important role during ripening include Lipases, proteinases. Chymosin is the major proteinasesin traditional animal rennets. The general pathways for the formation of volatile and nonvolatile compounds are well characterized for most cheese varieties, and detailed knowledge is available on the production of the primary products of lipolysis free fatty acids (FFA), glycolysis (lactate and products of citrate metabolism) and proteolysis free amino acids (FAA) in certain varieties (e.g., Gouda and Cheddar). However, much work remains to be done in order to understand the mechanisms by which these primary products are converted to volatile flavour compounds.

Keywords

Cheese Flavour, Flavour Compounds, Lipolysis, Proteolysis Metabolism of Lactate.

Introduction

Cheese is a biochemically dynamic product and undergoes significant changes during its ripening period. Freshly-made curds of various cheeses have bland and largely similar flavours. During the ripening period flavour compounds are produced which are characteristic for each variety [McSweeney and Sousa, 2000, McSweeney, 2004]. Cheese flavour resulted from a single compound or class of compounds, while this is largely true for blue-mould varieties (whose flavour is dominated by alkan-2 ones). It is now generally accepted that the flavour of most cheese results from the combination of a large number of several compounds present in the correct ratios and concentrations, which is known as component balance theory [Bosset and Gauch, 1993]. Cheese flavour development is a complex process in which enzymes from milk, rennet, starter cultures and secondary flora are involved in the degradation of milk proteins, fat and carbohydrates. Variations in non-starter lactic aid bacteria (NSLAB) and derived compounds depend on cheese variety, processing and ripening conditions [Novikova and Ciprovičas, 2009].

The flavour of fresh cheese, which is ready to be eaten immediately after manufacture, is the result of the action of starter bacteria and due largely to diacetyls and possibly acetaldehydes. The flavour of matured cheese is the result of the interaction of starter bacteria; enzymes from the milk; ripening from the rennet and accompanying lipases, and secondary flora [Urbach 1997].

Starter bacteria have a dual role in the production of cheese: acid production during manufacture and flavour development during ripening. Most rennet cheeses are ripened before consumption to achieve desirable organoleptic qualities. Ripening involves a series of complex biochemical processes, which can be grouped broadly into proteolysis, lipolysis and lactose/ lactate metabolism. The extent and type of ripening depend on storage time and temperature, cheese composition (especially moisture and salt levels) and the type and activities of enzymes and microorganisms present [Farkye and Fox, 1990].

Cheese flavour is one of the most important criteria determining consumer choice and acceptance. Cheddar cheese flavour varies widely with source, age, and fat content. However, aged Cheddar cheese flavor is characterized by sulfur, frothy, and nutty flavours [Urbach, 1997, Drake *et al*, 2001, Avsar *et al*, 2004]. The role of sulfur compounds in Cheddar cheese flavour [Milo and Reineccius, 1997] and their formation from sulfur containing amino acids by bacterial activity [Urbach, 1995, Weimer *et al*, 1999] or Strecker

degradation [Griffith and Hammond, 1989] have been investigated extensively and reviewed [Weimer *et al*, 1999]. Unlike sulfur flavour, knowledge on the nutty flavour of Cheddar cheese is scarce. First of all, defining the sensory term "nutty" appeared to be a difficult task, as the aroma quality in all nuts are not exactly the same [Clark and Nursten, 1977]. Drake *et al*, [2001] developed a defined sensory language for Cheddar cheese flavour. Nutty flavor was defined as the "(nonspecific) nut-like aromatic associated with different nuts." Lightly toasted unsalted nuts, unsalted wheat thins, or roasted peanut oil extract were used as references for nutty flavor. It is not clear whether nutty flavour is a product of a single compound or a combined effect of several compounds. Also, nutty character and the volatile source of nutty flavour may vary with different types of cheese [Clark and Nursten, 1977, Avsar *et al*., 2004]. The majority of studies on nutty flavor in cheese have been carried out on Swiss type cheese due to its distinct sweet and nutty notes. A range of compounds, such as ketones, lactones, esters, alcohols, aldehydes, pyrazines, sulfurous compounds, carbonyl compounds, free fatty acids, free amino acids, and salts have been reported to contribute to nutty flavor [Biede and Hammond, 1979a, 1979b, Liardon *et al*, 1982, Vangtal and Hammon, 1986, Warmke *et al*, 1996, Preininger *et al*, 1996, Rychlik and Bosset, 2001a]. Specifically, acetic and propionic acids, the major products of propionic acids bacteria, were claimed to play an important role in nutty flavours of this particular cheese type.

Cheese is the generic name for a group of fermented milk based food products. More than 500 varieties of cheeses are listed by the International Dairy Federation [IDF, 1982& Singh *et al.*, 2003] and numerous minor and/or local varieties also exist (Fox, 1987). The flavour profiles of cheeses are complex and variety- and type-specific. This was realized back in the 1950s, when [Mulder, 1952, Kosikowski and Mocquot, 1958] proposed the "component balance" theory. According to this theory, cheese flavor is the result of the correct balance and concentration of a wide variety of volatile flavor compounds.

The content of free amino acids (FAA) increases during ripening of cheese. Amino acids are the final products of casein breakdown performed by rennet, plasmin and lactic acid bacteria (LAB) and sometimes also other microorganisms in co-operation. The composition of free amino acids in cheese, however, only partly reflects the composition of casein, one explanation is that not all parts of casein are broken down equally and which parts are hydrolyzed depends on the cheese variety [Sousa *et al*, 2001, McSweeney 2004]. Another explanation is that the amino acids in cheese are the results of microbial activities in some amino acids are used and others may be produced and excreted into the cheese matrix.

Definition of Flavour

Flavour is the sensation produced by a material taken in the mouth, perceived principally by the senses of taste and smell, and also by the general pain, tactile, and temperature receptors in the mouth. Flavour also denotes the sum of the characteristics of the material which produces that sensation. Flavour is one of the three main sensory properties which are decisive in the selection, acceptance, and ingestion of a food.

Flavour is defined also as the complex sensation comprising aroma, taste and texture. Little is known about the nature of the aroma compounds, but it is clear that the breakdown products of lactose and citric acid (lactic acid**,** diacetyl, CO_2**,** etc.), of para casein (peptides and amino acids), and of lipids (free fatty acids) are essential for the flavour. A correct balance must exist between the various flavour substances. Lactic acid causes the refreshing acid taste, which is particularly noticeable in young cheese. An excess of lactic acid renders the cheese sour. Indirectly, lactic acid exerts influence on the texture of cheese. Large change in flavour developed during maturation. Numerous secondary products formed during the fermentation of lactose and the subsequent partial transformation of lactic **acid** affect aroma and taste (e.g. aldehydes, ketones, alcohols, esters, organic acids, CO_2). Proteolysis is also essential in flavour formation. Para casein is tasteless, but many degradation products are not; for example, peptides may be bitter and many amino acids have specific tastes, sweet, bitter or broth like, in particular. Short peptides and amino acids contribute at least to the basic flavour of cheese. Higher temperature, higher pH, higher water content and lower NaCl content in cheese appear to flavour proteolysis, in particular the formation of amino acids. Mature cheese contains numerous volatile compounds, usually in small amounts. These are predominantly degradation products of amino acids, e.g. NH_3, various amines, H_2S, phenyl acetic acid [Walstra *et al*, 1987, Carbonell *et al*, 2002, Vitova *et al*, 2006].

Several important flavour compounds of different types of cheeses are shown in Table 1. Since not all

products were analyzed by GC-O. Not all flavour components may be called key-flavours. The flavour compounds are categorized by the metabolic pathway/substrate they are most likely derived from, as will be discussed as an indication the following references are given: Limburger [Urbach, 1993], Gruye`re [Rychlik and Bosset 2001a , Rychlik and Bosset 2001b], Gorgonzola [Moio *et al.,* 2000], Mozzarella [Moio *et al,* 1993], Parmigiano [Bosset and Gauch 1993 , Qian and Reineccius 2002], Grana Padano [Moio and Addeo 1998], Maho´n, Fontina, Comte´, Beaufort and Appenzeller [Bosset and Gauch 1993].

Role of Starter during Ripening

Information to verify the function of starter on flavour production of cheese dose not completely describes the mechanisms for production of the full flavour of mature cheese or if the lactic bacteria had a major direct role in forming the flavour.

The functions of the starter bacteria are : (1) the production of lactic acid, which together with the rennet causes the curd to form, acts as a preservative and contributes to the acid flavour of the cheese, (2) metabolism of citric acid, which is widely regarded being essential for flavour production, (3) breakdown of the protein (in conjunction with the rennet and enzymes from milk), (4) some contribution to the breakdown of the diglycerides formed from the milk triglycerides by the lipoprotein lipase from the milk (5) the breakdown of hippuric acid to benzoic acid, [Sieber *et al*, 1990] which acts as a natural preservative. Traditionally, mixed or undefined strain starter cultures were used which composed a number of strains of *Lactococcus lactis subsp. Cremoris* or the closely related *lactococcus lactis sub sp. Lactis.* Species of lactic acid bacteria which were able to metabolize citrate were sometimes present in the mixed strain starter cultures [Cogan and Hill, 1995].

TABLE 1 EXAMPLES OF IMPORTANT FLAVOUR COMPONENTS IN SOME TYPES OF CHEESE (SMIT *ET AL.* 2005)

Metabolism	**Gouda**	**Cheddar**	**Camembert**	**Swiss – type (and Massdam)**
Amino acid	3- Methylbutanal 3- Methylbutanol Methanethiol Dimethylsuphide (DMS) 2- Mehtylpropanol Dimethyltrisuplphied (DMTS)	3- Methylbutanal Isovaleric acid Methional Methanethiol DMDS DMTS	3-Methybutyrate 3-Methylbutanal Methional Methanethiol DMs Benzaldhyde Phenylacetaldehyde	Methional 3-Methylvutanal Skatole
Sugar	Diacelyl	Propionic acid Diacetyl	2.3- Butanedione	Propionic acid Diacetyl
Fat	Butyric acid Butanon Hexanal Pentanal	Butyric acid Acetic acid 1-Octen-3-one Butanone	1- Octen-3-ol Butyric acid 1-Octen-3-one 2-Undecalactone γ-Decalactone	
Rest and combined pathways	Ethyl butyrate Limonene	Ethyl butyrate Ethyl hexanoate	Phenylethyl acetate	Ethyl butyrate Ethyl hexanoate Ethyl -3-mehtylbutanoate Phenylethyl acetate
References	[Neeter and De Jong 1992], [Engels 1997]	[Christensen and Reineccius 1995], [Curioni and Bosst 2002]	[Kubickova and Grosch 1997]	[Curioni and Bosset 2002], [Prieninger and Grosch 1994]

However some negative aspects of these cultures were occurred, resulting in variation in the rate and level of acid production and they could cause undesirable open texture in the cheese. A defined strain system was developed to overcome these problems [Lawreene and pearce,LL, 1972]. However, the strains are selected

primarily on their ability to produce acid and little attention has been focused on their flavour-generating capacity during ripening. The complete role of starters in the development of Cheddar flavour has not been fully elucidated [Crow *et al*,1993, Quintans *et al*, 2008] However, due to the high starter cell numbers reached during cheese manufacture, it is to be expected that starter strains and their enzymes do play an important role in flavour development. Corynebacterium variabile is part of the complex microflora on the surface of smear-ripened cheeses and contributes to the development of flavor and textural properties during cheese ripening. Still little is known about the metabolic processes and microbial interactions during the production of smear-ripened cheeses [Schröder *et al*, 2011].

Role of Enzymes during Ripening

Enzymatic processes are responsible for the production of a considerable number of compounds which, as a result of their presence, concentration and proportions, are often characteristic of particular cheese types [Sable and Cottenceau, 1999]. The influence of the native milk flora on the flavour and texture of raw milk cheese is still not well known. Cheese made from raw milk tends to develop a stronger and more specific flavour than cheese made from pasteurized milk and generally ripens more quickly. Changes in cheesemilk caused by pasteurization include denaturation of indigenous enzymes, slight denaturation of whey proteins and their interaction with caseins and the destruction of thermolabile member of the indigenous microflora. [Bachman *et al*, 1996, Quintans *et al*, 2008]

Lipases in cheese originate from 6 sources: the milk, rennet preparation (rennet paste), starter, adjunct starter, nonstarter bacteria and if used, extensive lipases. The origin of lipases in varieties characterized by exogenous lipolysis is usually from the coagulant or from the adjunct starter (mould ripened cheeses) [Mc Sweeney and Sousa, 2000, Mc Sweeney 2004]. The coagulants used to clot milk and crud preparation of selected proteinases which often possess a considerable proteolytic activity. Chymosin is the major proteinase in traditional animal rennets (88-94 % milk clotting activity, MCA), with the remainder pepsin [Roth *et al*, 1977]. The principal role of chymosin (or other coagulants) in cheese making is to specifically hydrolyze the phe^{105} - Met ^{106} bond of the micelles-stabilizing protein, κ-casein, during the coagulation of milk. Most of coagulant activity added to the milk is lost in the whey, but about 6% is retained in the curd depending on factors including coagulant type, cooking temperature and pH at drainage; residual coagulant contributes to proteolysis in many varieties [Creamer *et al*, 1985]. In high cooked cheese (e.g., Emmental), chymosin in denatured extensively and makes relatively little contribution to ripening [McSweeney and Sousa, 2000, Mc Sweeney, 2004].

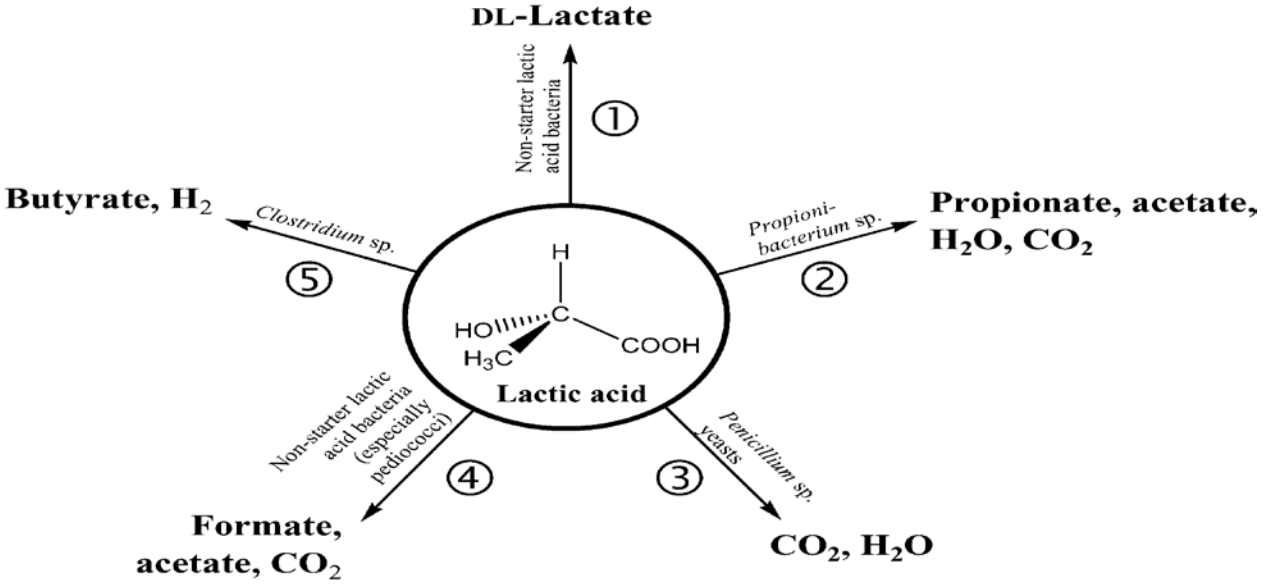

FIG. 1 PATHWAYS BY WHICH LACTATE IS METABOLIZED IN CHEESE DURING RIPENING. 1) RACEMIZATION, 2) METABOLISM BY PROPIONIBACTERIUM FREUDENREICHII IN SWISS CHEESE, 3) OXIDATIVE METABOLISM OF LACTATE, 4) CONVERSION TO FORMATE, ETHANOL AND ACETATE AND 5) ANAEROBIC METABOLISM OF LACTATE TO BUTYRATE AND H2, WHICH LEADS TO LATE GAS BLOWING (REPRINTED FROM CHEESE: CHEMISTRY, PHYSICS AND MICROBIOLOGY, VOL 1 (3RD EDITION) FOX P F, MCSWEENEY P L H, COGAN T M & GUINEE T P (EDS). MCSWEENEY P L H & FOX P F METABOLISM OF RESIDUAL LACTOSE AND OF LACTATE AND CITRATE, PP 361–371, COPYRIGHT 2004, WITH PERMISSION FROM ELSEVIER).

Biochemical Pathways during Cheese Ripening

Metabolism of Lactose, Lactate and Citrate

Lactose metabolism to lactate is essential to the production of all cheese varieties. Depending on starter type, lactose is metabolized by the glycolytic (most starter bacteria) or phozontributes to the flavour of ripened cheese varieties, particularly early in maturation. Acidification of the cheese has a major indirect effect on flavour, since it determines the buffering capacity of the cheese and thus the growth of various microorganisms during ripening and the activity of the enzymes involved in cheese ripening. Depending on variety, lactate may also be further metabolized by a number of pathways to various compounds which contribute to cheese flavour [Fig.1].

The production of D-lactate during ripening is probably greater in cheeses made from raw milk [Steffen *et al*, 1980]. Racemization of lactate has little impact on flavour but may have undersirable nutritional consequences, particularly for infant. The solubility of Ca-D-lactate is less than that of Ca-L-lactate, and Ca-D-lactate may crystallize in cheese forming white specks, particularly on cut surfaces [Fox *et al*, 1990].

Lactate can be oxidized in vitro to acetate and CO_2 by components of the non-starter lactic bacteria (NSLAB) present in hard cheeses [Fox, *et al*, 1995b] as shown in [Fig.2].

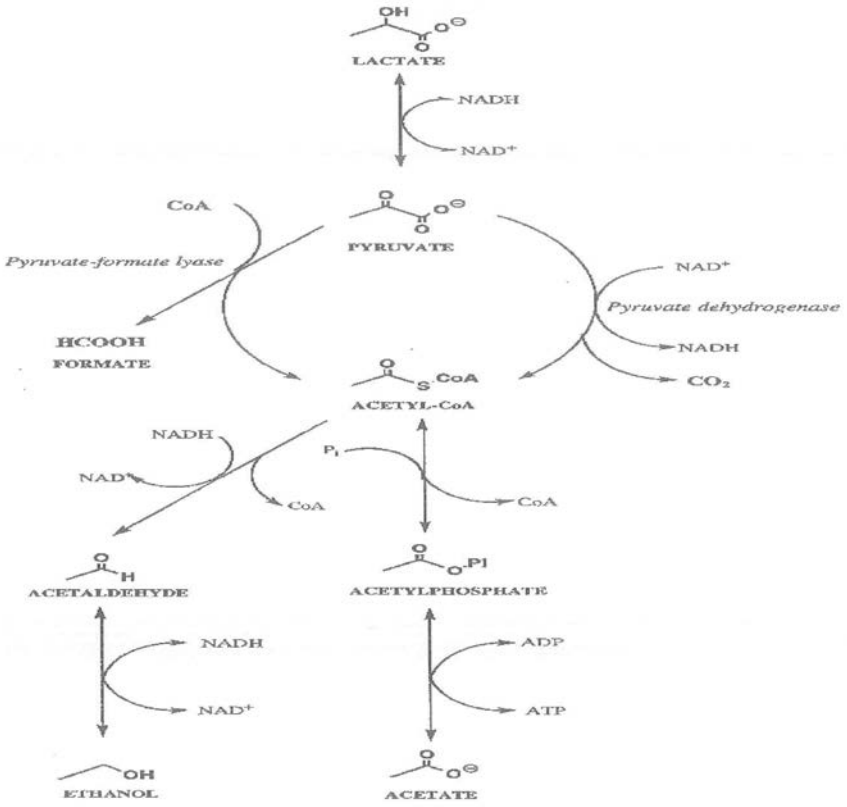

FIG. 2 METABOLISM OF LACTATE BY LACTOCOCCI (MODIFIED FROM FOX P.F., SINGH T.K., MCSWEENEY P.L H., BIOGENESIS OF FLAVOUR COMPOUNDS IN CHEESE, IN: MALIN E.L., TUNICK M.H. (EDS.), CHEMISTRY OF STRUCTURE/FUNCTION RELATIONSHIPS IN CHEESE, PLENUM PRESS, NEW YORK, PP. 59–98, COPYRIGHT 2004, WITH PERMISSION FROM ELSEVIER).

Acetate, an important flavour compound in many cheeses, in addition to being formed from lactose by lactic acid bacteria (LAB) may also be formed as a result of citrate and lactate metabolism, or as a product of the catabolism of amino acids.

In case of swiss-type cheeses *propionibacterium SP*. Metaboliz L- lactate to propionate, acetate and CO_2. The carbon dioxide produced is essential for eye development; and propionate and to a lesser extent, acetate contribute to the flavour of these cheeses. Fermentation of lactose and lactate in swiss-type cheeses has been described by [Steffen *et al*, 1987].

In Camembert and Brie (surface mould-ripened cheese) the metabolism of lactate is most important

[Karahadia and Lindsay, 1987]. The mesophilic starter bacteria produce lactic acid in the curd about 1% which is quickly metabolizes by secondary microorganisms. The yeasts and moulds rapidly metabolize lactate to CO_2 and H_2O,and the pH of the cheese surface increases when the lactate has been exhausted, *P.camemberti* metabolizes amino acids released from the casein with the production of NH_3 [Gripon, 1993].

Milk contains about 8 mmol/L^{-1} citrate, most of which is lost in the whey during cheese making, since about 94% of the citrate is insoluble phase of the milk. Nevertheless, the low concentration of citrate in cheese curd (10 mmol/kg) is a great importance since it may be metabolized to a number of volatile flavour compounds by certain mesophilic starters (citrate positive, cit $^{+}$, *lactococci and leuconostoc sp*.) by pathways summarized in [Fig. 3].

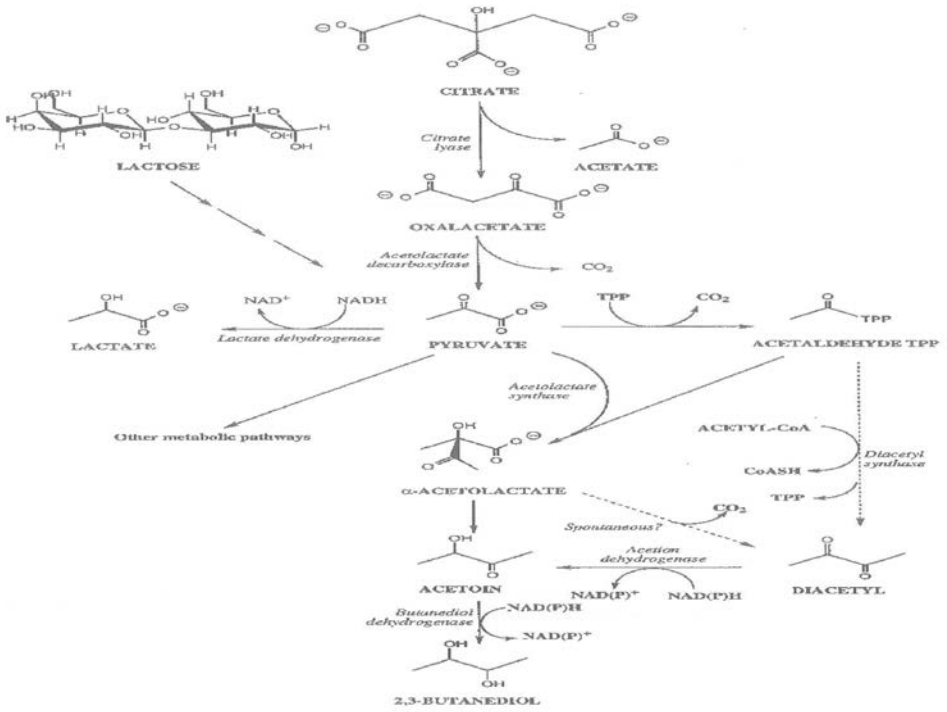

FIG. 3 PATHWAYS FOR CITRATE-POSITIVE STRAINS OF LACTOCOCCUS AND *LEUCONOSTOC SP.* (REPRINTED FROM CHEESE: CHEMISTRY, PHYSICS AND MICROBIOLOGY, VOL 1 (3RD EDITION) FOX P F, MCSWEENEY P L H, COGAN T M & GUINEE T P (EDS). MCSWEENEY P L H & FOX P F METABOLISM OF RESIDUAL LACTOSE AND OF LACTATE AND CITRATE, PP 361–371, COPYRIGHT 2004 WITH PERMISSION FROM ELSEVIER).

Citrate metabolism has been reviewed by several researchers [Cogan and Hill 1993, Fox *et al*, 1990, Hugenholtz, 1993]. Cit^{+} microorganisms do not utilize citrate as an energy source, but rather it is co-metabolized with lactose or some other sugar.

The principal flavour compounds produced on metabolism of citrate are acetate, diacetyl, acetoin and 2, 3-butanediol. Diacetyl is usually produced only in small amount **(**1-10 μg/ ml in milk), but acetoin is generally produced in much higher quantities **(**10-50 fold higher than diacetyl concentrations**)**. Acetate is produced from citrate in equimolar concentrations. Production of 2, 3- butanediol by starters has not been studies in detail, despite its importance, the exact reactions which result in the formation of diacetyl remain unclear. Diacetyl could be produced directly from acetaldehyde-thiamine pyrophosphate (TPP) and acetyl- COA by enzymic action, but diacetyl synthase has never been identified clearly in LAB.

Acetoin is produced from α-acetolactate by the action of acetolactate decarboxylase. Products of citrate metabolism produced by pure cultures of Cit^{+} *lactococci and leuconostoc sp*. differ: the former produce diacetyl, acetoin and CO_2 in addition to lactate, but the latter produce large amounts of lactate and acetate.

Acetate is produced from acetylphosphate with the concomitant production of 1 mol ATP, resulting in faster growth of the microorganisms. In mixed cultures, *leuconostoc sp*. Produced diacetyl and acetoin perhaps because their ability to take up lactose is

greatly reduced below pH 5.5.

Citrate metabolism is of particular importance is responsible for eye formation. Diacetyl is an important aroma compound in a number of varieties including Dutch- type cheese, Quarg and Cottage cheese. Diacety can be converted to acetoin and 2, 3-butanediol and 2 butanone which are also important flavour compounds is some cheese varieties [Dimos *et al,* 1996].

Lipolysis and Metabolism of Fatty Acids

Milk fat is essential for the development of the correct flavour in cheese during ripening. Cheddar and other cheeses normally made from whole milk do not develop correct flavour when made from skim milk or milks in which milk fat has been replaced with other lipids [Wijesundera *et al,* 1998, Mc Sweeney, 2004]. Indeed, satisfactory flavour development is one or the principal problems encountered in the manufacture of reduced- fat variants of established cheese varieties.

Lipolysis is particularly extensive in hard Italian verities, surface bacterially-ripened (smear) cheese and blue mould chesses and is essential to correct flavour development in these cheeses. Extensive lipolysis is considered undesirable in other types of cheese varieties such as Cheddar, Gouda and Swiss cheeses; high levels of fatty acids in these cheeses lead to rancidity. However, low concentrations of FFA contribute to the flavour of these cheeses, particularly when they are correctly balanced with the products of proteolysis or other reactions [Bosset and Gauch, 1993, Rychlik *et al*, 1997].

Rennet extracts used in the production of most cheese varieties should be free from lipase activity, but rennet pastes used to coagulate the milk for certain Italian varieties (e.g., Provolone, Romano) contain pregastric esterase (PGE) which is responsible for the extensive lipolysis in these cheeses [Nelson *et al,* 1977].The lipase esterase system of starter bacteria has received much less attention than their proteolytic system lactococcus sp. Are only weakly lipolytic, but may be for liberation of quite high levels of FFA when present in high cell numbers or over extended ripening periods. Lipases/ esterases of lactococcus strain, which appear to be intracellular, have been studied [Chik *et al,* 1997, Fox and Wallace, 1997, hallond and Coolbea, 1996). Obligately homofermentative *lactobacilli* used as starters (*Lb. helveticus, Lb. delbrueckii subsp. bulgaricus and Lb. delbrueckii subsp. lactis*) also produce esterases, some of which have been studied e.g., [El- Soda *et al*, 1986, Khalid and Marth, 1990]. Facultatively heterofermentative lactobacilli (e.g., *Lb. casei, Lb. paracasei and Lb. plantarum),* which dominate the NSLAB flora of many cheese varieties, are weakly lipolytic. *Micrococcus* and *Pediococcus* are also weakly lipolytic [Bhownik and Marth, 1990]. Psychrotrophic bacteria (e.g., *Pseudomonas sp*.) produce heat-stable lipases which adsorb onto the fat globules in milk and survive pasteurization. They may contribute to lipolysis in cheese made from milk containing high numbers of psychrotrophic bacteria prior to pasteurization [Cousins *et al*. 1977]. *Penicillium sp.* produces potent extracellular lipases which are primarily responsible for the extensive lipolysis in mould-ripened cheeses [Gripon 1993 and Smit *et al.* 2005]. The impact of FFA on the flavour of blue mould-ripened cheeses is less than for hard Italian varieties, possibly due to neutralization as the pH increases during ripening, and because of the dominant influence of methyl ketones on the flavour of blue mould cheeses.

Furthermore, FFA acts as precursor molecules for a series of catabolicreactions which lead to the production of other flavour compounds (Fig. 4). The flavour of blue mould cheeses is dominated by alkan-2-ones (2-methyl ketones).

The pathway by which alkan-2-ones are produced (β-oxidation) involves the release of fatty acids by lipolysis, their oxidation to β- ketoacids and decarboxylation to alkan-2-ones with one less C-atom. Akan-2- ones may be reduced to the corresponding secondary alcohols (alkan-2-ols), a step which is reversible under aerobic conditions. The production of alkan-2-ones in blue mould cheese has been discussed by some researchers [Gripon, 1993, McSweeney *et al,* 1995, McSweeney, 2004, Molimard and Spinnle, 1996].

Lactones are cyclic compounds formed by the intramolecular esterification of hydroxy fatty acids. The principal lactones in cheese are γ- and δ- lactones which have 5- and 6-sided rings, respectively, and are stable, strongly flavoured and could be formed from the corresponding γ – or δ-hydroxy fatty acids. [Urbach, 1993] reported that in fullfat cheeses δ -decalactone increased to a maximum at about 14 weeks and then decreased, whereas in low-fat cheeses, the level of δ -decalactone remained fairly constant throughout ripening. However, the fact that the Cheddar cheese flavour actually improved when the δ -decalactone level decreased may indicate that δ – decalactone plays very little part in Cheddar cheese flavour [Dimos *et al,* 1996]. Hydroxylation of fatty

acids can result from the normal catabolism of fatty acids, and/or they can be generated from unsaturated fatty acids by the action of lipoxygenases or hydratases [Dufosse *et al,* 1994]. FFA can react with alcohols to yield esters (which are highly flavoured) or with free sulphydryl groups to give thioesters.

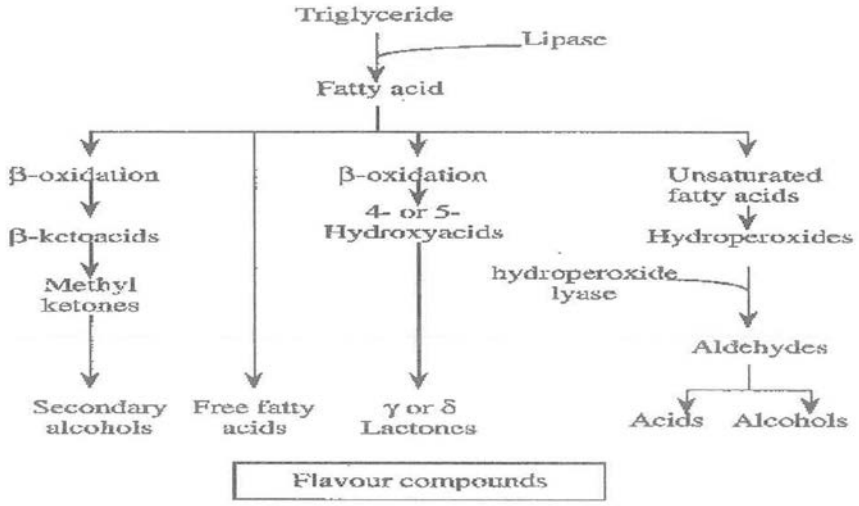

FIG. 4 GENERAL PATHWAYS FOR THE CATABOLISM OF FREE FATTY ACIDS IN CHEESE [MCSWEENEY AND SOUSA 2000].

Fourteen different esters have been found in Emmental [Bosset *et al,* 1995 and 1997, Imhof and Bosset, 1994, Rychlik *et al,* 1997] and esters have also been claimed to be important contributors to the flavour of Parmigiano-Reggiano [Meinhart and Schreier, 1986].

Proteolysis

Proteolysis during cheese ripening is the most complex and important events which occurred and has been discussed by several reviews [Fox and law 1991, Fox and McSweeney, 1996, Fox *et al.,* 1995b].

Proteolysis plays a vital role in the development of : (1) textural changes in the cheese curd, due to breakdown of the protein network, decrease in water activity through water binding by liberated carboxy and amino groups and increase in pH (in particular in surface mould –ripened varieties) (2) direct contribution to flavour and perhaps to off-flavour (e.g., bitterness) of cheese through the formation of peptides and free amino acids, (F.A.A); (3) liberation of substrates (amino acids) for secondary catabolic changes (e.g., deamination, decarboxylation, transamination, desulphuration catabolism of aromatic compoubnds such as phenylalanine, tyrosine, tryptophane and reactions of amino acids with other compounds); and (4) changes to the cheese matrix, which facilitate the release of the flavoured aromatic compounds. The methodology for assessment of the extent and pattern of proteolysis in cheese is of interest as an index of cheese maturity and quality, and has also been reviews [Fox and Law1991 Fox *et al,* 1995 a, Fox and McSweeney, 1996].

During ripening, proteolysis in cheese is catalyzed by enzymes from: (1) the coagulant (e.g., chymosin, pepsin, or plant or fungal acid proteinases); (2) the milk (plasmin, cathepsin D and perhaps other somatic cell proteinases); (3) the starter; (4) the nonstarter; or (5) the secondary starter (e.g., *P. camemberti, P. roqueforti, Propionibacterium spp., Br. linens* and other coryneforms); and (6) exogenous proteinases and/or peptidases used to accelerate ripening.

In most cheese varieties, the initial hydrolysis of caseins is caused by the coagulant and to a lesser extent by plasmin and perhaps somatic cell proteinases (e.g., cathepsin D), which results in the formation of large (water-insoluble) and intermediate-sized (water-soluble) peptides which are subsequently degraded by the coagulant and enzymes from the starter and non-starter flora of the cheese. The production of small peptides and FAA is caused by the action of microbial proteinases and peptidases.

The final products of proteolysis are FAA, the concentrations of which depend on the cheese variety, and which have been used as indices of ripening [McSweeney and Fox, 1997]. The concentration of FAA in cheese at any stage of ripening is the net result of the liberation of amino acids from casein and their transformation to catabolic products. The principal amino acids in Cheddar cheese are Glu, Leu, Arg, Lys, Phe and Ser [Wijesundera *et al,* 1998]. Concentrations of amino acids generally increase during ripening, with the exception of Arg., the concentration of which

is reported to decrease later in ripening [Puchades *et al*, 1989]. The level of peptides and FAA soluble in cheese in 5% phosphotungstic acid (PTA) has been considered to be a reliable indicator of the rate of flavour development [Ardo and petterson, 1988] and the composition of the amino acid fraction and the relative proportions of individual amino acids are thought to be important for the development of the characteristic flavour [Broome *et al*, 1990].

Medium and small peptides and FAA contribute to the background flavour of most cheese varieties [Urbach, 1995] and some individual peptides have brothy, bitter, nutty and sweet tastes. Fox and Wallace, [1997] have suggested that flavour the concentration of FAA could not be correlated, since different cheeses (e.g., Cheddar, Gouda and Edam) have very different flavours, although the concentration are relative proportion of FAA are generally similar.

Catabolism of Amino Acids

Catabolism of free amino acids (FAA) can result in a number of compounds, including ammonia, amines, aldehydes, phenols, indole and alcohols, all of which may contribute to cheese flavour. Catabolism of FAA probably plays some role in flavour development in all varieties, but it is particularly significant in mould and smear-ripend cheese [Fox *et al*, 1995 b]. The first stage in amino-acid catabolism involves decaboxylation, deamination, transaminatio, desulphuration or perhaps hydrolysis of the amino-acid side-chains. The second stage involves conversion of the resultion compounds (amines and α- ketoacids), as well as amino acid themselves, to aldehydes, primarily by the action of deaminases on amines, the final stage of amino- acid catabolism is the aldehydes to alcohols, or their oxidation to acids. Sulphur-containing amino acids can undergo-exrensive conversion, leading to the formation of a number of compounds, including methanethiol and other sulphur derivatives [Fox and Wallace, 1997]. General pathways for the catabolism of FAA are summarized in [Fig. 5].

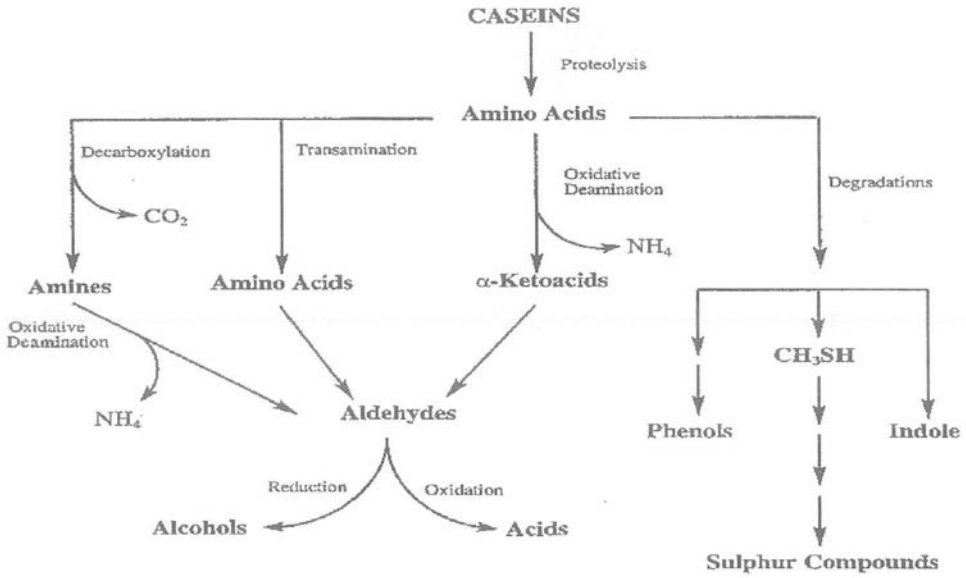

FIG. 5 GENERAL PATHWAYS FOR THE CATABOLISM OF FAA (MODIFIED FROM [HEMME *ET AL.* 1982 AND MCSWEENEY AND SOUSA 2000].

Esterases and Acyltransferases

Esters, such as ethylbutyrate, contribute to Cheddar and Gouda flavour, although, an excess of esters in proportion to other flavour compounds could be responsible for the fruity defect of Cheddar [Bills *et al*, 1965]. In Camembert, phenylacetaldehyde, 2-phenylethanol and the ester phenylethyl acetate, which all result from phenylalanine degradation, are identified in fractions with floral rose-like odour [Kubickova. and Grosch, 1997], and could cause the pleasant floral note of this cheese [Roger *et al*, 1988]. Esters are formed in a reaction between an alcohol and an organic acid [Engels and Visser, 1994, Acree *et al*, 1984]. Besides amino acid metabolism, also sugar and fat metabolism provide substrates for ester formation

[Molimard and Spinnler, 1996, Yvon and Rijnen, 2001]. Although ester formation is generally considered to be an enzymatic catalysed reaction, the reaction between acetyl-CoA and methanethiol is spontaneous [Helinck *et al*, 2000]. Esterases and lipases are serine hydrolases capable of synthesizing or hydrolysing esters, depending on the environmental conditions, while alcohol acetyltransferases only catalyse ester synthesis. By knocking out the esterase gene (estA) in L. lactis, [Fernandez *et al*, 2000] showed that all ester hydrolysing activity in L. lactis was lost and that this organism most probably had only one enzyme with esterase activity. Later, this EstAenzyme has been reported to be responsible for the formation of short chain fatty acid esters in vitro [Nardi *et al*, 2002]. The extrapolation of these data to cheese is not directly possible, since the reaction equilibrium for these kinds of esterifications depends strongly on environmental parameters like water activity. The degradation of thio-esters and ethyl esters by EstA was also observed at conditions resembling the cheese matrix indicating that EstA is capable of degrading thio-esters and ethyl esters at cheese-like conditions.[Liu *et al*, 2004] described an alternative reaction for formation of esters by dairy lactic acid bacteria. The process, alcoholysis, is essentially a transferase reaction in which fatty acyl groups and acyl groups from acyl-CoA derivatives are directly transferred to alcohols.

Bitterness and Other off-Flavour

Bitterness in cheese is due mainly to hydrophobic peptides and is genrally regarded as a defect, although bitter notes may contribute to the desirable flavour of mature cheese. The literature concerning bitterness in dairy products has been reviewed by [Lemieux and Simard, 1991, 1992], [McSweeney *et al*, 1997, Singh *et al*, 2003]. Certain sequences in the caseins are particularly hydrophobic and, when excised by proteinases, can lead to bitterness. The action of coagulant has been implicated in the formation of bitter peptides in cheese, and thus factors that affect the retention and activity of rennet in the curd may influence the development of bitterness. The starter and rennet type are considered important in the development of bitterness. [Lawrence *et al*, 1972] have suggested that the major role of rennet in the development of bitterness may be the production of long peptides that will be subsequently degraded to small bitter peptides by starter proteinases.

Off-flavours (rancidity) can be due to excessive or unbalanced lipolysis caused by lipases/ esterase from starter or non-starter LAB, enzymes from psychrotrophs in the cheese milk, or indigenous milk lipoprotein lipase.

Late gas blowing and off-flavours in certain hard cheese result from the metabolism of lactate (or glucose) by *Clostridium sp.* To butyric acid and H_2 [Fox *et al*, 1995b], these defects may be avoided by good hygiene, addition of NO_3 or lysozyme, or by the physical removal of spares by bactofugation or microfiltration.

Carbon dioxide produced by citrate fermentation can cause undesirable openness and the defect in Cheddar and Cottage cheeses.

In Cheddar, fruity flavour is regarded as a defect by professional cheese graders, although consumers may be prepared to pay a premium for fruit Cheddar [McSweeney and Sousa 2000].

Application of Strains with Selected Enzyme Activities for Improving Cheese Flavour

For the application of selected lactococci, it was found that strains possessing a specific flavour-forming enzyme do not necessarily possess other enzymatic activities of the complete pathway. In addition, strains might lack other characteristics for application as cheese starter (e.g., fast acidification). In order to be able to use such strains and to overcome problems, it is required to combine selected strains with industrial strains in order to obtain a starter with both good flavour generating potential as well as good acidifying and proteolytic activities [Ayad *et al*, 2000 and Ayad *et al*, 2001a].

It was found by [Ayad *et al*, 2001b] that different strains could influence each other in formation of flavour components. Strains, each of which has only a limited set of enzymes in a certain pathway could complement each other. For instance, the combination of L. lactis B1157 and SK110 strains in milk resulted in the formation of high levels of 3-methylbutanal. In SK110, a highly proteolytic strain from industrial origin, the complete pathway from casein via leucine to 3-methylbutanal cannot proceed due to the lack of a decarboxylating enzyme. L. lactis strain B1157, on the other hand, is a non-proteolytic wild strain and thus unable to produce enough free amino acids that can serve as substrate for the subsequent transamination and decarboxylation steps. However, when B1157 and SK110 are cultivated together, the strains complement each other with regard to their enzyme activities

resulting in a high production of the chocolate flavour component 3-methylbutanal. This proto-cooperation between strains as it is called offers new possibilities for the construction of tailor-made starter cultures, because it makes clear that not all the desired enzyme activities in a certain flavour pathway lead to flavour present in one strain.

An example of the application of knowledge of proto-cooperation, but also of population dynamics of starter cultures for the optimisation of a cheese flavour is given by [Ayad *et al*, 2002 and Smit *et al*, 2005]. A selected L. lactis strain (strain B851) with high (in vitro) activity to form 3-methylbutanal was used to improve the taste of Proosdij cheese. Proosdij cheese is a Gouda-type cheese, prepared with a mesophilic starter culture in combination with a thermophilic adjunct culture. This cheese has a flavour profile, which has characteristics between Gouda and Parmesan cheese. One of the key flavour components in this type of cheese is 3-methylbutanal [Engels, 1997 and Neeter and De Jong, 1992]. The selected L. lactis strain B851 was used in combination with the regular cultures used for this type of cheese. The cheeses made with and without the selected adjunct strain were analysed for the production of 3- methylbutanal by headspace gas chromatography [Ayad *et al*, 2001b] and graded by an expert panel [Ayad *et al*, 2003]. It was found that the use of the selected adjunct strain in cheese resulted in both an increase in the keyflavour production as well as in the intensity of the Proosdij cheese flavour.

In order to control the flavour intensity, the selected strain was first tested at different levels in a defined strain starter (DSS) culture as well as in combination with a mixed strain starter (MSS) culture. The latter is generally used for Gouda and Proosdij-type cheese production. The results of population dynamics, sensory evaluation and analysis of volatile compounds pointed to the possibility of controlling both the cell numbers of strain B851 as well as the flavour intensity resulting from this strain in cheese. Based on these results, B851 was used to enhance the flavour development of a Proosdij-type cheese made with a new thermophilic culture B1138. This culture has previously been developed to prevent crack formation in Proosdij cheese. In this cheese, the addition of culture B851 led to an increase in the overall flavour intensity, indicating that it is possible to tailor the flavour of cheese by using specifically selected cultures, even in combination with complex starter cultures [Smit *et al.* 2005 and Mikelsone& Ciprovica 2011].

Conclusion

The general pathways for the formation of volatile and nonvolatile compounds are well characterized for most cheese varieties, and detailed knowledge is available on the production of the primary products of lipolysis (FFA), glycolysis (lactate and products of citrate metabolism) and proteolysis (FAA) in certain varieties (e.g., Gouda and Cheddar). However, much work remains to be done in order to understand the mechanisms by which these primary products are converted to volatile flavour compounds. Medium and small peptides and FAA contribute to the flavour of most cheese varieties. Catabolism of free amino acids (FAA) can result in a number of compounds, including ammonia, amines, aldehydes, phenols, indole and alcohols, all of which may contribute to cheese flavour. Off- flavour may be due to:

- Excessive or unbalanced lipolysis.
- Lat gas blowing and off-flavour in certain hard cheeses result from the metabolism of lactate (or glucose).
- CO_2 produced by citrate fermentation can cause undesirable openness.

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